Accuracy of heparin-induced platelet aggregation test for the diagnosis of heparin-induced thrombocytopenia

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Summary

Introduction

Whereas the utility of washed platelet assays such as the heparin-induced platelet activation test (HIPA) for the diagnosis of heparin-induced thrombocytopenia (HIT) is regarded as high, the performance of simpler assays such as the heparin-induced platelet aggregation test (PAT) is still elusive. Using well-characterized samples of a large cohort study, we aimed to assess the diagnostic accuracy of PAT for the diagnosis of HIT.

Material and Methods

One-hundred twenty-two immunoassay-positive serum samples of a previous, prospective single-center cohort study including consecutive patients with suspected HIT (n=1291) were used. HIPA was determined as reference gold standard; samples were previously analyzed using PAT as well as polyspecific platelet factor 4/heparin enzyme-linked immunosorbent assay (ELISA). 4Ts score was calculated using the patient documentation. Diagnosis of HIT was defined as a positive HIPA, which is a positive reaction in 2 out of 4 donor platelets within 30 minutes.

Results

HIT was diagnosed in 39 out of 122 patients corresponding to a prevalence of 32%. Median optical density (ELISA) was 2.8 (inter-quartile range 2.3, 3.0) in patients with HIT and 0.7 (0.5, 1.3) in patients without HIT. PAT was positive in 27 out of 39 HIT patients and it was negative in 83 out of 83 HIT-negative patients. Thus, the sensitivity of PAT for the diagnosis of HIT was 69% (95% confidence interval 52%, 83%) and the specificity 100% (96%, 100%).

Conclusions

Our results demonstrate that PAT is a valuable test to confirm HIT but cannot be applied to rule-out HIT in clinical practice.

Key words: Heparin-induced thrombocytopenia;
Heparin/adverse effects;
thrombocytopenia/chemically induced;
thrombocytopenia/diagnosis;
immunoassay/methods;

Introduction

Diagnosing heparin-induced thrombocytopenia (HIT) is challenging. A number of diagnostic tests are available, but all are associated with certain drawbacks [1, 2]. Clinical assessment tools like the 4Ts score can be applied during weekends and nightshifts, but these tests are observer-dependent, and the diagnostic accuracy is clearly restricted [3]. Immunoassays such as platelet factor 4[PF4]/heparin enzyme-linked immunosorbent assay (ELISA) are able to demonstrate anti-heparin/PF4 antibodies with a high sensitivity [1], but they can hardly differentiate antibodies which activate platelets and other that do not [4-6]. In addition, the diagnostic accuracy varies according to type of antibody specificity, thresholds, and manufacturer. Platelet-activating antibodies, which are able to cause clinical HIT, can be demonstrated using functional assays only [4, 7].

Washed-platelet assays, such as serotonin release assay (SRA) or heparin-induced platelet activation test (HIPA) are regarded as gold-standard tests for the diagnosis of HIT [8]. However, SRA and washed-platelet HIPA are complicated, time-consuming and require a highly specialized laboratory [8]. Thus, results are rarely available in a timely manner [1]. A number of other functional assays have been developed to overcome these limitations. The first functional test for HIT was the heparin-induced platelet aggregation test (PAT) done on platelet-rich plasma (PRP) [1, 9]. PAT done on PRP is a promising assay because it can be conducted using light transmission aggregometers, which are available in many laboratories, and utilizing PRP, which avoids platelet-washing procedures and handling of radioactive agents [4]. A number of authors proposed and used PAT done on PRP for diagnosis of HIT [9-13]. However, the diagnostic accuracy of PAT done on PRP for HIT is still not fully established [1].

Using well-characterized samples of a large single-centre cohort study, we aimed to assess the diagnostic accuracy of PAT done on PRP for the diagnosis of HIT.

Methods

Design, setting and population

The design of the study is shown in Figure 1. We used patient data and remaining samples of a previous single-centre cohort study [10], in which 1291 consecutive patients were included, representing a mixed population (53.5% internal medicine, 23.5% surgery including cardiac surgery, and 20.1% intensive care unit). Median age was 67.9 years (inter-quartile range [IQR]: 1.5-106.4) and 43.8% of the patients were female. Inclusion criteria were “suspected HIT” corresponding to a requested HIT test or a requested consultancy service (4Ts score obtained). Clinical data were obtained as well prospectively as retrospectively (4T’s score in some patients), and a number of laboratory tests were conducted: particle gel immunoassay (ID-H/PF4 PaGIA; DiaMed SA, Cressier sur Morat, Switzerland), polyspecific heparin/PF4-ELISA (GTI Diagnostics, Waukesha, WI, USA), and PAT done in PRP (conducted in all patients). The detailed characteristics with regard to patients, setting, determination of laboratory tests, and collection of clinical data are described in detail elsewhere [10].

For the purpose of the present investigation, we randomly selected 122 patient samples with remaining serum or plasma samples and positive immunoassay out of the study cohort mentioned above, the inclusion criteria was a positive immunoassay (optical density [OD] of ELISA ≥ 0.4 and/or positive PaGIA). Washed-platelet HIPA was conducted between March and June 2017. Presence of HIT was defined as a positive washed-platelet HIPA, determined as described below. The number of samples drawn from the previous cohort study was based on practicability issues and availability of remaining samples rather than a full power analysis. The study was approved by the appropriate ethical committee (#1900476).

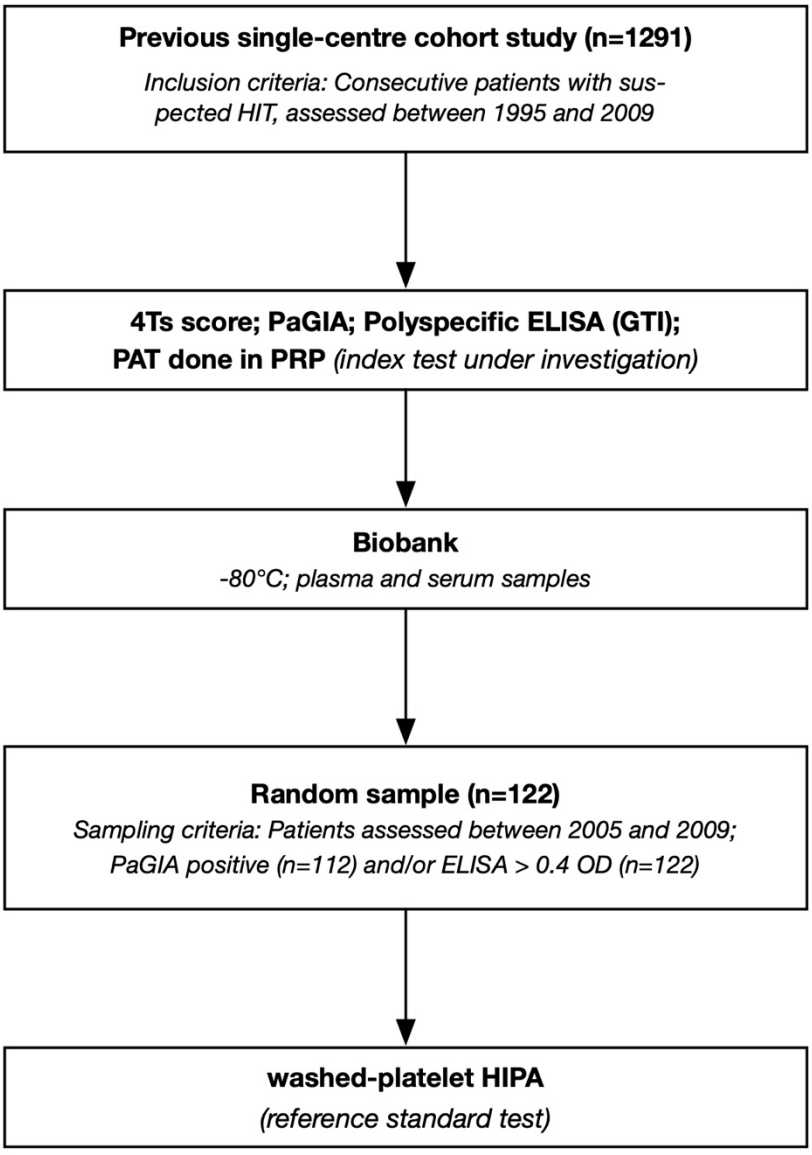

Figure 1 |Design of the current cohort study. Abbreviations: HIT, heparin-induced thrombocytopenia; HIPA, washed platelet heparin-induced platelet activation test; PAT, heparin-induced platelet aggregation test done on platelet-rich plasma; PaGIA, particle gel immunoassay (PaGIA); OD, optical density.

Determination of PAT done on PRP

PAT done on PRP was conducted as previously described [9]. Briefly, PRP of four selected donors was incubated with platelet poor plasma of the patient in presence of three different heparin solutions: 0.5 U/ml UFH, 0.5 U/ml low molecular weight heparin (LMWH; nadroparin), as well as 100 U/ml unfractioned Heparin (UFH Liquemin®). Similar to previous publications, four healthy donors were selected in order to account for accidental effects that inhibit or stimulate platelet activation [9, 12]. Donors were selected if previous aggregation studies demonstrated platelet aggregation in presence of HIT-sera. Light transmission was determined using an aggregometer (APACT 4004, LABiTec, Ahrensburg, Germany) for 15 minutes. PAT done on PRP was considered as positive if aggregation was at least 50% in two out of four selected donors (using low concentrations of UFH or LMWH respectively), and if the reaction could be suppressed by high concentrations of UFH.

Collection and storage of samples

A standardized protocol for blood withdrawal was implemented to ensure adequate pre-analytic conditions. Plasma samples were collected into 3.2% sodium citrate (10mL, S-Monovette, Sarstedt Nümbrecht, Germany). Platelet-poor plasma was obtained by double centrifugation at 1500x g for 10min at room temperature. Serum and plasma samples were snap-frozen and stored in polypropylenes tube at -80°C [10].

Preparation of samples and determination of washed-platelet HIPA

To generate washed platelets, whole blood of five unselected healthy donors was collected into acid-citrate-dextrose solution (ACD) to prevent platelet aggregation and to bind residual calcium [4, 11]. PRP was produced by a low speed centrifugation scheme without break (120g for 20 minutes) and platelets were washed in two steps. First, the platelet pellet was re-suspended in calcium and magnesium-free Tyrode's buffer at pH 6.3 adding glucose and apyrase. The second pellet was re-suspended using calcium- and magnesium-containing Tyrode's buffer at pH 7.2. Afterwards, platelet suspension was incubated at 37°C for 45 minutes [4, 8, 11].

Patient's samples were thawed for 5 minutes at 37°C and heated at 56°C for 45 minutes to inactivate residual thrombin. The following substances were added into the designed wells of a 96-well microplate: 10 µL of suspension buffer, a low concentration of LMWH (Reviparin) (0.2U/ml), a high concentration of UFH (Liquemin®) 100 U/ml, 20 µL patient's serum, and 75 µL platelet's suspension. Thereafter the microplate was incubated for 45 minutes on a magnetic stirrer plate, stirring two steel balls per well at high speed (600 rotations per minute). Platelet activation was observed by monitoring translucent transformation. The test is considered positive if platelet activation occurs in at least 2 out of 4 platelet donors using LMWH but not UFH after not more than 30 minutes [4, 8, 11].

Statistical analysis

Descriptive statistics were used to characterize the study population and describe the distribution of test results (median and inter-quartile range [IQR]/ ranges or frequencies as

appropriate). Two-by-two tables were generated by stating true positives, false positives, false negatives, and true negatives. Diagnostic accuracy of PAT done on PRP was determined by calculating the sensitivity, the specificity and the likelihood ratio in relation to the presence of HIT (defined as a positive washed platelet HIPA test). Analyses were performed using the Stata 14.1 statistic software package (StataCorp. 2014. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Results

One-hundred twenty-two patient samples with a positive immunoassay test result were analyzed. Detailed patient characteristics according to the presence of HIT are shown in Table 1. Median age was 70 years, IQR 60 to 77. Fifty-one of the patients were female (41.8%). ELISA was positive in all patients and PaGIA was positive in 112 patients.

Presence of HIT was confirmed by a positive washed-platelet HIPA in 39 patients, the prevalence in the cohort was 32%. Median 4Ts score was 4.5 in patients with HIT (IQR 4, 5), and 3 in patients without HIT (IQR 2 to 4). Median OD of ELISA was 2.8 in patients with HIT (IQR 2.3, 3.0) compared to 0.7 in patients without (IQR 0.5, 1.3).

Figure 2 shows the contingency table illustrating the diagnostic accuracy of PAT done on PRP. The sensitivity was 69% (95% CI 52%, 83%) and the specificity 100% (95% CI 96%, 100%). The corresponding positive likelihood ratio was 116 (95% CI 7, 1846) and the negative likelihood ratio 0.3 (95% CI 0.2, 0.5).

	HIT positive n=39 (32%)	**HIT negative** n=83 (68%)	**All patients** n=122 (100%)
Age in years, median (IQR)	70 (62, 77)	68 (55, 77)	70 (60, 77)
Sex, numbers (%)			
Male	21 (17.2)	50 (40.1)	71 (58.2)
Female	18 (14.8)	33 (27.0)	51 (41.8)
4T's score, median (IQR)	4.5 (4, 5)	3 (2, 4)	4 (3, 5)
Polyspecific GTI ELISA, median OD (IQR)	2.84 (2.30, 3.01)	0.71 (0.53, 1.34)	1.12 (0.62, 2.32)
PaGIA, median titre (IQR)	8 (4, 32)	0 (0, 2)	1 (0, 8)

Table 1 |Baseline characteristics of the current study cohort (n=122). A random sample of patients with a positive ELISA or PaGIA result respectively was drawn out of the population of a previously conducted large cohort study [8]. Presence of HIT was defined as a positive heparin-induced platelet activation test (washed-platelet HIPA). Abbreviations: HIT, heparin-induced thrombocytopenia; IQR, interquartile range; OD, optical density; ELISA, enzyme-linked immunosorbent assay; PaGIA, particle gel immunoassay

PAT done on PRP *(Index test)*	**HIT** *(Numbers of patients)*			
	Positive	**Negative**	Total	**Prevalence** *32%*
Positive	27	0	27	**PPV** ***100%*** *(95% CI 77%,100%)*
Negative	12	83	95	**NPV** ***87%*** *(95% CI 81%, 92%)*
Total	39	83		
	Sensitivity **69%** *(95% CI 52%, 83%)*	**Specificity** **100%** *(95% CI 96%, 100%)*	**LR+** **116** *(95% CI 7, 1846)*	**LR-** **0.3** *(95% CI 0.2, 0.5)*

Figure 2 |Contingency table illustrating the diagnostic accuracy of PAT done on PRP for the presence of HIT. Presence of HIT was defined as a positive washed-platelet HIPA assay. Abbreviations: HIT, heparin-induced thrombocytopenia; PAT done on PRP, heparin-induced platelet aggregation test; washed-platelet HIPA, heparin-induced platelet activation test using washed platelets; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, negative likelihood ratio

Discussion

Using samples and patient data of a previous, prospective single-center cohort study, we observed a clearly inferior sensitivity of PAT done on PRP compared to washed-platelet HIPA. In contrast, specificity was excellent. However, in view of the high risk of thromboembolism in patients with a missed diagnosis of HIT, a test with a limited sensitivity is not useful in clinical practice.

Even though previous investigations differ in characteristics of the patients and samples, and determination of PAT done on PRP vary among centers, our results are in-line with previous investigations. Chong and colleagues reported a sensitivity between 65% and 94% depending on the reactivity of the platelets [12]. Sensitivity of PAT done on PRP was clearly inferior to washed-platelet HIPA in 209 samples of patients with suspected HIT [11]. In accordance with

our results, all patients with a positive PAT done on PRP results had a positive washed-platelet HIPA result as well. In a study done by Favaloro and colleagues in 140 selected samples, sensitivity of PAT done on PRP was inferior to SRA but specificity was high [13].

The strength of our investigation is that we studied 122 samples of well-characterized patients, included in a previous prospective cohort study [10]. Only immunoassay-positive samples were included, resulting in a comparable high prevalence of 32%. Thus, the present cohort constitutes an appropriate representation of the target population of a functional assay for HIT. Our study has limitations as well. First, the samples were stored at -80°C for several years. Even though pilot studies did not indicate significant changes in antibody activity over time, we cannot fully exclude minor effects. Second, the number of samples used was more based on practicability issues and the availability of residual material rather than a full power analysis. However, with a view on the confidence intervals determined, we do not believe that a larger study population would have changed the results. Third, data on clinical endpoints supporting or contradicting the diagnostic classification as done by HIPA are not available within the present database.

In conclusion, our results confirm that PAT done on PRP is an appropriate test to verify a HIT-positive sample for scientific purposes. However, the sensitivity is clearly limited and it is not possible to rule-out HIT in clinical practice.

Addendum

MN designed the study, analysed the data, and wrote the manuscript. JB conducted the analyses, analysed the data, and wrote the manuscript. LA designed and collected data of the previous cohort study, implemented the biobank, and wrote the manuscript. AAS contributed to implementation of HIPA, provided infrastructure, interpreted the analysis, and wrote the manuscript. All authors approved the final version of the manuscript.

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Declaration of interest

All the authors state that they have no conflict of interest.

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